THE NON-EXISTENCE OF 'PROTAGON' AS A DEFINITE CHEMICAL COMPOUND.

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In 1865 Liebreich[1] obtained a crystalline substance from brain by means of a simple extraction process (either with ether alone, or with alcohol after preliminary treatment with ether). He considered it to be the mother-substance of all the bodies previously isolated from brain; these bodies were regarded by him as decomposition products of the mother-substance, to which he therefore gave the name of *Protagon*. Liebreich disregarded the results of previous workers in this field. As early as 1834 Couerbe[2] had prepared and analysed a substance which he called *cérébrote*; this was obtained from brain by a process of which Liebreich's was merely a modification. Liebreich's views were at first generally accepted[3], but were discarded a few years later mainly on account of Diaconow's work[4]. Diaconow considered protagon to be a mixture of cerebrin and lecithin. Thudichum (1874) was the first to show the true nature of protagon; he proved that it was not a definite chemical compound and that it consisted of a mixture of substances, several of which he separated out in crystalline form. In 1879, however, protagon was rehabilitated by the investigations of Gamgee and Blankenhorn[5], which seemed to

[1] *Liebig's Annalen*, CXXXIV. p. 29. 1865.

For a complete history of the subject see Thudichum, *Die chem. Konstitution des Gehirns des Menschen u. der Thiere*, Tuebingen, 1901, and Posner and Gies, *Journ. of Biol. Chem.* I. p. 59. 1905.

[2] *Annales de chimie et de physique*, LVI. p. 160. 1834.

[3] No doubt the fact that protagon shows a microcrystalline structure influenced chemists to accept it as a uniform chemical substance. This fact also had been described before Liebreich's observations by v. Bibra (*Vergl. Unters. ü. d. Gehirn*, etc., 1854).

[4] *Centralbl. f. d. med. Wissensch.* VI. p. 97. 1868.

[5] *Zeitschr. f. physiol. Chem.* III. p. 260. 1879. See also Gamgee, *Physiol. Chem. of the Animal Body*, p. 427. London, 1880.

corroborate those of Liebreich. Thudichum's objections, based on laborious work, were disregarded and from that time protagon was again considered to be a definite chemical compound by subsequent workers (Geoghegan, Parkus, Baumstark, Kossel and Freytag, Ruppel, Zuelzer, Noll). Kossel and Freytag's[1] work really confirmed the results of Thudichum, but instead of accepting his views they explained the varying phosphorus percentage of their substances by assuming the existence of various protagons. In 1900 Wörner and Thierfelder[2] also noticed the impossibility of obtaining a substance of uniform phosphorus percentage on repeated recrystallisations of protagon, and Koch[3] (1902) as well as Lesem and Gies[4] (1902) obtained similar results. These researches threw considerable doubt on the uniformity of protagon, until the work of Cramer[5] (1904) in Liebreich's laboratory, once more lent credence to it.

Under these circumstances a reinvestigation of the whole question seemed desirable, especially as the method already published by one of us[6] for the preparation of cholesterin from brain afforded a ready means for the systematic investigation of the other chemical constituents of brain. While our work was in progress Posner and Gies[7] published a paper dealing with the same subject. Their results, obtained by somewhat different methods, agree with ours and confirm Thudichum's views.

We have prepared protagon by Liebreich's method (as modified by Gamgee and Blankenhorn and also by Cramer) and by a new method in which acetone instead of alcohol is used as the solvent. We have further prepared cérébrote according to Couerbe's directions so as to investigate its identity or otherwise with protagon. In order to determine its constancy of composition we have relied on phosphorus and nitrogen estimations before and after repeated recrystallisations under the conditions described by the several authors. We further subjected protagon to fractional crystallisations at different temperatures (the method employed by Thudichum) and with different solvents. We have also, as a further test of uniform composition, investigated the optical activity and the amount of galactose formed on hydrolysis.

For convenience of reference we give in the following table the

[1] *Zeitschr. f. physiol. Chem.* XVIII. p. 431. 1893.
[2] *Ibid.* XXX. p. 542. 1900.
[3] *Ibid.* XXXVI. p. 134. 1902.
[4] *Amer. Journ. of Physiol.* VIII. p. 183. 1902.
[5] This *Journal*, XXXI. p. 30. 1904.
[6] O. Rosenheim. This *Journal*, XXXIV. p. 104. 1906.
[7] *Loc. cit.*

composition and method of preparation of protagon by different workers.

TABLE I.

	C	H	N	P	Short description of method
Couerbe's cérébrote (1834)	67·82	11·10	3·40	2·33	Extraction of brain with cold ether, followed by repeated extraction with boiling alcohol. Cérébrote separates from alcoholic extract on cooling. Not recrystallised.
Liebreich's protagon (1865)	66·74	11·74	2·80	1·23	Extraction with cold ether and water at 0° C. followed by extraction with alcohol at 45°. Alcoholic extracts deposit protagon when placed on ice. Recrystallised from alcohol.
Gamgee & Blankenhorn's protagon (1879)	66·30	10·46	2·29	1·03	Repeated extraction with alcohol at 45°. The protagon is deposited on cooling and freed from cholesterin by ether. Recrystallised three times.
Cramer's protagon (1904)	66·35	10·98	2·29	1·04	Brain is treated with a sodium sulphate solution at 100° and protagon extracted from the coagulum by boiling alcohol. It is afterwards freed from cholesterin by ether. Twice recrystallised.

1. *Couerbe's Cérébrote.*

It will be seen from the above table that Couerbe's cérébrote represents in reality a crude protagon. The only serious modification in the process of its preparation introduced by Liebreich consists in the extraction with alcohol at 45° C. instead of at its boiling point and in subsequent recrystallisation of the product. The reason for the choice of this temperature of 45° is Liebreich's statement that protagon seems to be decomposed at higher temperatures. This fear is, however, quite unfounded as has been shown by Thudichum and acknowledged by Gamgee. Cramer, the latest worker to uphold the entity of protagon, has used boiling alcohol again for its preparation.

We prepared cérébrote exactly according to Couerbe's directions, using sheep brains. The raw product was subsequently freed from cholesterin by extraction with ether in a Soxhlet's apparatus. It was then three times recrystallised from boiling 85% alcohol. The following results were obtained on analysis[1] (see Table II.).

[1] All the substances were dried *in vacuo* and the analyses were carried out in duplicate. Phosphorus was estimated by Neumann's method and nitrogen by Kjeldahl's.

TABLE II.

Analysis of Couerbe's Cérébrote.

	1st preparation		2nd preparation[1]	
	P	N	P	N
Crude product, cholesterin free	1·09	4·34	1·02	4·43
Once recrystallised	0·68	2·21	—	—
Thrice recrystallised	0·65	2·08	—	—
Insoluble residue of third recrystallisation	0·60	2·24	—	—
Solids in mother liquid of third recrystallisation	0·99	—	—	—

[1] The high nitrogen figures of the first preparation were thought to be due to particles of brain tissue which had passed through the muslin used for filtration. In the second preparation therefore a perfectly clear filtrate was obtained by filtration through paper.

It will be seen that the phosphorus figures of the crude product are considerably lower than Couerbe's, but agree well with those of protagon. The nitrogen on the other hand is higher; on recrystallisation a certain amount of the crude product did not dissolve, and this portion, removed by filtration, showed a very high nitrogen percentage, whereas in the recrystallised portion the nitrogen percentage is markedly lower. Boiling alcohol seems therefore to extract a highly nitrogenous substance, an observation which was also made with the preparation of protagon according to Cramer, although the previous coagulation of the proteins by sodium sulphate in this process no doubt excludes the possibility of any admixture of protein.

In its behaviour towards solvents and in its physical characters (crystalline form, etc.) cérébrote closely resembles protagon, and these facts taken together with the similarity of their method of preparation leave no doubt that we have to deal with the same substance under two different names.

2. *Gamgee and Blankenhorn's Protagon.*

Gamgee and Blankenhorn modified Liebreich's method by omitting the treatment of the brain with ether and water at 0° C. before the alcohol extraction at 45°C. on account of the difficulty of separating the ether from the swollen brain tissue. The product, which was deposited on cooling the alcoholic extracts, was subsequently treated with ether to free it from cholesterin. As this method has practical advantages it was adopted by all later workers.

We prepared Gamgee and Blankenhorn's protagon from ox brain

following their instructions in detail. The crude product, free from cholesterin, was recrystallised from 85 % alcohol at 45°. The following figures were obtained on analysis.

Analysis of Gamgee and Blankenhorn's Protagon.

	P	N
Crude protagon, cholesterin free[1]	1·18	3·79
Recrystallised protagon	0·91	2·49

[1] In a second preparation the extracts were filtered through paper instead of muslin, in order to avoid admixture of brain tissue. This crude protagon gave the following figures on analysis P = 1·07 %, N = 2·23 %.

The crude protagon, prepared according to Gamgee and Blankenhorn, evidently resembles Couerbe's cérébrote in its nitrogen and phosphorus percentage very closely. The recrystallised protagon shows a slightly lower phosphorus percentage than that of Gamgee and Blankenhorn, whose analytical figures for phosphorus were also slightly lower than Liebreich's.

This recrystallised substance (which according to Gamgee and Blankenhorn represents pure protagon) was subjected to fractional crystallisation. Forty grammes were suspended in 4 litres of 85 % alcohol. The contents of the flask were kept at 45° for 5 hours, being stirred mechanically during the whole time. On filtration 6·7 g. remained on the filter as undissolved protagon. The filtrate was cooled on ice when 26 g. were deposited and subsequently removed by filtration. The mother liquor on evaporation yielded 2·9 g.

TABLE III.

Showing the products of fractional crystallisation obtained from once crystallised protagon by treatment with 85 % alcohol at 45°.

	P %	N %	P : N
Original protagon once recrystallised	0·91	2·49	1 : 6
I. Fraction at 0° = twice recrystallised protagon	0·94	2·20	1 : 5
II. Insoluble fraction at 45°	0·19	1·87	1 : 22
III. Fraction from mother liquid	2·28	—	

It will be seen that in this process of fractional crystallisation which evidently cannot effect any serious chemical decomposition, this sample of pure protagon furnished fractions of varying phosphorus and nitrogen percentage. The relationship P : N also undergoes marked changes. The insoluble fraction from which Thudichum separated a phosphorus-

free substance, which he called "Phrenosin[1]," has always been neglected by workers on protagon, as has been the case with the phosphorus-rich one kept in solution after the filtration of the fraction called protagon. But there can be no doubt that they formed part of the original protagon, which has been considered as a body of definite chemical constitution.

The substance obtained as fraction I. in the above treatment, and which represents twice recrystallised protagon, was now subjected to fractional crystallisation with acetone at different temperatures. It had been noticed in some earlier experiments that acetone formed a suitable solvent for protagon. Its relatively low boiling point (56°) and chemical inertness allow of its use at boiling point without fear of subjecting the protagon to any chemical decomposition. It was further noticed that the solutions of protagon in acetone began to deposit a precipitate on cooling at about 40° which increased until the temperature had fallen to 30°. This precipitate settled down rapidly and was easy to filter. The solution filtered at 30° kept perfectly clear until the temperature fell to about 25°—20°, when a further precipitate formed, which was quite different in physical appearance, being somewhat gelatinous and very difficult to filter. The appearance of the dried products was also quite different, the one obtained at 30° being a white powder, that deposited at a lower temperature a yellowish, somewhat waxy substance[2].

[1] Gamgee (see *Textbook*, *loc. cit.* p. 441) has also obtained from this residue a substance practically free from phosphorus, which he termed "pseudocerebrin." Thierfelder (*Zeitschr. f. physiol. Chem.* XLIII. p. 21. 1904) has acknowledged that his "cerebron," which he extracted from protagon by means of such solvents as benzene or chloroform mixed with alcohol, is identical with Gamgee's pseudocerebrin. He obtained from it by acid hydrolysis the same products which Thudichum had previously obtained from phrenosin, namely galactose, cerebronic acid (Thudichum's neurostearic acid) and sphingosine, a base already described by Thudichum. Lately Kitagawa and Thierfelder (*ibid.* XLIX. p. 286. 1906) stated that they obtained by hydrolysis in methyl-alcoholic solution another base, and therefore doubt the uniformity of sphingosin. They seem to have quite overlooked the fact that Thudichum also obtained at least one other base, called by him psychosine, from the products of acid hydrolysis in ethyl-alcoholic solution, and that this base, when further treated with acid in aqueous solution, is split into sphingosine and cerebrose (galactose). Koch (*Amer. Journ. of Physiol.* XI. p. 310. 1904) pointed out that Thudichum's phrenosin and Thierfelder's cerebron (Gamgee's pseudocerebrin) are identical. Posner and Gies (*loc. cit.*) urge the retention of the name phrenosin given to this substance by Thudichum, who was the first to isolate it. In view of the evident identity of phrenosin and cerebron as shown by their methods of preparation, properties and hydrolytic products, we are also of opinion that Thudichum's original term phrenosin should be retained.

[2] Very similar observations had already been made by Thudichum, who used, however, alcohol as a solvent. His results were confirmed by Posner and Gies.

Twenty grammes of the twice recrystallised protagon (Gamgee and Blankenhorn) were subjected therefore to a quantitative fractional crystallisation at various temperatures and the resulting products analysed ; 2½ litres of acetone were used.

The results are shown in the following table.

TABLE IV.

Showing the fractional products obtained from pure twice recrystallised protagon by acetone at different temperatures.

	Weight in grs.	P %	N %	P : N
Original protagon (twice recrystallised)	20·00	**0·94**	2·20	1 : 5
I. Protagon fractions				
(i) at 38°	1·74	**1·40**	2·54	1 : 4
(ii) at 20°	3·29	**0·21**	1·81	1 : 18
(iii) at −5°	0·35	**0·34**[1]	—	—
II. Insoluble protagon	12·09	**1·05**	2·41	1 : 5
III. Solids in the mother liquid	?[2]	**0·49**[1]	—	—

[1] One analysis only.

[2] Through an accident some of this was lost.

The results of this experiment show clearly that from pure (twice recrystallised) protagon substances of widely different phosphorus and nitrogen percentages may be separated by means of fractional crystallisation, a method excluding any chemical decomposition. From a substance containing 0·94 % of phosphorus, fractions were obtained by one operation which contained as much as 1·40 % and as little as 0·21 % of phosphorus. It is clear from this result alone that we have to deal here not with a uniform single chemical substance, but with a mixture of substances.

Nevertheless it might conceivably be argued that the twice recrystallised protagon employed for this experiment was not yet quite pure and that the fractions showing a higher or a lower phosphorus and nitrogen percentage are impurities, the main fraction representing the real protagon with 1·05 % phosphorus and 2·41 % nitrogen. This specimen of protagon had so far only been subjected to recrystallisation by means of the two solvents alcohol and acetone. Before studying the effect of another solvent this main fraction was again recrystallised from alcohol at 45° and the recrystallised product was treated with acetone at 45°. On analysis it showed now a phosphorus percentage of 1·16.

One gramme of this thrice recrystallised protagon was now treated with a mixture of 20 c.c. methyl-alcohol and 5 c.c. chloroform[1], in which

[1] Kitagawa and Thierfelder (*loc. cit.*) also made use of mixtures of methyl-alcohol and chloroform for the isolation of cerebron (phrenosin) from protagon.

it dissolved easily at 55°. At room temperature a large amount crystallised out showing on analysis 0·58 % phosphorus, and the mother liquid left on careful evaporation a white powder containing 1·87 % phosphorus. The results are shown in the following table.

TABLE V.

Showing the fractional products obtained from thrice recrystallised protagon by methyl-alcohol containing 20 % *chloroform.*

	Weight in grs.	P %
Original thrice crystallised protagon	1·00	1·16
Fraction obtained at 15°	0·57	0·58
Fraction from mother liquid	0·37	1·87

From the result of this experiment it was to be expected that the original crude protagon (p. 5) would also furnish on recrystallisation from a similar solvent a substance much poorer in phosphorus than protagon. An experiment justified this *a priori* conclusion. Absolute alcohol containing 20 % chloroform was used as a solvent and the protagon, showing an initial phosphorus percentage of 1·18, gave on recrystallisation a substance with only 0·58 % phosphorus. This experiment furnishes the most striking proof for the composite nature of protagon. By mere recrystallisation from a solvent different from those hitherto applied, the recrystallised product shows a decrease of 50 % in its phosphorus, while the phosphorus in the more soluble fraction rises by over 60 %.

3. *Cramer's Protagon.*

Cramer explained the discrepancies of the results of Wörner and Thierfelder and of Lesem and Gies by assuming that their preparations of protagon were contaminated with the substance called by Gamgee pseudocerebrin (Thudichum's phrenosin, Thierfelder's cerebron, see note on p. 6). As these observers however had prepared their protagon exactly according to the classical methods, it follows that all protagons prepared before them must have been likewise contaminated. Moreover Cramer himself made no attempt to exclude in his own preparations this "contamination." He did not subject his protagon to any process of fractional crystallisation and has in no way proved that his protagon was not a mixture.

Cramer made use in his method of an observation communicated to him by Liebreich that protagon is "coagulated" by boiling it with

salt solutions[1]. He therefore heated the finely minced brain with sodium sulphate solution on the water-bath and subsequently extracted it with *boiling* alcohol, thus reverting to Couerbe's method. The protagon, deposited from the alcoholic extracts by cooling on ice, was freed from cholesterin by ether and recrystallised from boiling alcohol. The substance thus obtained was regarded as pure protagon and analysed. (See Table I.)

We prepared Cramer's protagon from sheep brains. The finely minced brain was heated in a boiling water-bath with 5 % sodium sulphate solution and subsequently extracted six times with boiling alcohol, following Cramer's directions in every detail. The final product, freed from cholesterin, was three times recrystallised from boiling alcohol. It presented the physical properties of a typical protagon.

The following table shows the results of analysis.

TABLE VI.

Showing the phosphorus and nitrogen percentages of protagon prepared by Cramer's process.

	P %	N %
Crude protagon	0·91	3·06
First crystallisation	0·91	2·15
Third crystallisation	0·79	2·34

The figures obtained for the crude Cramer's protagon, compared with those of our specimen of Couerbe's cérébrote (Table II.), show a great resemblance, as was to be expected from the similarity in the method of preparation. The phosphorus percentage is a little lower than that of Cramer's published analysis[2], and this is still further decreased by the third recrystallisation. From the method of its preparation it represents, however, a very pure protagon.

This thrice crystallised protagon was further subjected to a fractional crystallisation from acetone at different temperatures as described before.

Ten grammes were digested with 3 litres of boiling acetone, and an insoluble residue was subsequently removed by filtration. The filtrate was then kept at 36° C. for many hours and the precipitate which had settled was separated at that temperature. The liquid was now further

[1] It has been shown by Posner and Gies that this "coagulation" does not affect the chemical composition of protagon and that the main effect of the hot salt solution is merely to coagulate the proteins of the brain.

[2] In another preparation 1·07 % phosphorus was found.

cooled at 10° when a precipitate, this time of a gelatinous nature, was deposited and filtered at that temperature. The results obtained on analysis are given below.

TABLE VII.

Showing the fractional products obtained from Cramer's pure protagon by acetone at different temperatures.

	Weight in grs.	P%	N%
Original protagon (thrice recrystallised)	10·00	0·79	2·34
I. Insoluble protagon	5·20	0·96	1·93
II. Protagon fractions			
(i) at 36°	1·79	1·09	2·43
(ii) at 10°	1·36	0·32	1·62[1]
III. (i) from concentrated mother liquid	0·24	0·32[1]	—
(ii) solids from final mother liquid	0·51	0·13[1]	—

[1] One analysis only.

The same conclusions must be drawn as in the case of Gamgee and Blankenhorn's protagon. The results show that Cramer's pure protagon is a mixture of substances containing varying amounts of phosphorus and nitrogen.

4. *Protagon prepared by Extraction with Acetone.*

It has already been remarked in a former communication (*loc. cit.*) on the preparation of cholesterin from brain that the method then described (dehydration by means of plaster of Paris and subsequent extraction with cold acetone) seemed to be a convenient one for a systematic study of the brain constituents. It was found that after the complete removal of cholesterin by means of cold acetone, a substance was extracted by boiling acetone which in physical properties closely resembled protagon[1], and this was obtained initially as a snow-white powder, free from cholesterin and necessarily free from lecithin and kephalin, as the two latter substances are insoluble in acetone. When allowed to deposit slowly from its hot solutions, it assumed the characteristic crystalline forms of protagon.

In order to study its composition a large quantity was necessary and its preparation was directly combined with a fractional precipitation at different temperatures. For this purpose one quarter of the total extract obtained in each extraction was set apart and cooled on ice. The precipitates thus obtained were filtered on a filter surrounded by

[1] Cf. C. Tebb. This *Journal*, xxxiv. p. 106. 1906.

an ice-jacket and represented the unfractionated protagon. The remaining three quarters of each of the extracts were allowed to cool slowly and were filtered at different temperatures as described before and the fractions thus obtained analysed separately.

Nine ox brains, weighing 3150 grammes, were finely minced and mixed with 9500 grammes plaster of Paris. The mass was left over night and subsequently broken up into a coarse powder. This was extracted in a specially constructed apparatus at a temperature of 10°—15° with 8 litres acetone. The extracts were concentrated by distilling off the acetone on the water-bath, and the extraction repeated until the residue obtained from 8 litres only weighed 0·7 grammes and gave no reaction for cholesterin. Altogether, 12 extractions were made and 102·4 gr. raw cholesterin were obtained. (The last few extracts contained very little cholesterin, but a small amount of a waxy hygroscopic substance containing phosphorus, which has not yet been further examined.) After the extraction of the cholesterin by cold acetone was complete, the whole apparatus was heated, by means of a current of hot water flowing through an outer jacket, to a temperature of 56°, the boiling point of acetone, for 4 hours. The apparatus was arranged so as to allow of filtration at a temperature of 56°.

TABLE VIII.

Showing the composition of protagon obtained by acetone extraction and its fractional products at different temperatures.

	Protagon obtained from one quarter of the extract cooled on ice				Protagon fractions obtained from three quarters of the extract at different temperatures			
	Weight in grs.	P %	N %		Weight in grs.	Temp.	P %	N %
First extraction.								
Fraction I	7·19	1·06	1·69	I*a*	19·47	18°	0·96	2·29
				I*b*	1·21	0°	1·47	—
Second extraction.								
Fraction II	3·55	1·15	2·11	II*a*	6·37	30°	1·29	2·21
				II*b*	2·49	20°	0·57	1·80
				II*c*	3·00	0°	1·09	2·12
Third and fourth extractions.								
Fraction III	1·68 + 1·08 = 2·76	1·33	2·36	III*a*	2·53 + 1·54 = 4·07	30°	1·47	2·51
				III*b*	3·23 + 2·01 = 5·24	0°	1·00	1·96
Total	13·50			Total	41·85			

Solids from mother liquids: weight 20·60 grs., P % 2·35, N % 2·26.

The product obtained by acetone extraction of ox brain represents

in appearance and composition a typical protagon. Nevertheless as will be seen from the above figures, it consists of a mixture of substances showing various phosphorus and nitrogen percentages. As these fractions have been obtained at different temperatures from the identical solution which on cooling on ice deposited the typical protagon, there can be no doubt that they existed as such in solution and are not products of decomposition.

An interesting point is the increase in percentage of phosphorus and nitrogen in the later stages of the extraction with acetone. The more soluble phosphorus free-part of the protagon (phrenosin) seems to be extracted first, while the substances rich in phosphorus remain behind. The reverse takes place if alcohol is used as a solvent. In that case the less soluble part is poorer in phosphorus, thus indicating that it contains a preponderance of cerebrosides (phrenosin, kerasin). This behaviour of the protagon mixture towards the two solvents suggests a method for the preparation of its phosphorus-rich constituent (Thudichum's sphingomyelin) which we are at present engaged in isolating.

In a subsequent experiment protagon was prepared by the same method from human brain and the separate extracts were directly subjected to fractional crystallisation at different temperatures. The brain weighed 1210 grs. Twelve acetone extractions, each with 2½ litres, were made at 10°—15° until the last extract on evaporation left a residue weighing only 0·24 grs., which was free from cholesterin. The subsequent extracts were made at 56°. The results are given in the following table.

TABLE IX.

Products obtained from protagon by fractional crystallisation at different temperatures.

	Filtered at	Weight in grs.	P %	N %
First extraction.				
1st Fraction	30°	4·21	1·13	2·23
2nd ,,	0°	4·37	0·82	1·92
Second and third extractions.				
3rd Fraction	30°	2nd 2·90, 3rd 2·91 } 5·81	1·54	2·50
4th ,,	0°	2nd 2·76, 3rd 1·49 } 4·25	1·06	2·03
Solids from mother liquids		1st 2·95, 2nd 2·38, 3rd 1·63 } 6·96	2·36	2·27

The same remarks apply to these results as in the former case.

Further, we prepared protagon by the acetone process (dispensing however with the plaster of Paris) from human brain and recrystallised some of the products obtained by different extractions, from boiling acetone, estimating phosphorus in the insoluble as well as in the recrystallised substance. The following results were obtained.

TABLE X.

Showing the phosphorus percentages of protagon obtained by acetone extraction.

	1st	2nd	3rd—8th	9th—20th
Extraction at	45°	50°	56°	56°
Phosphorus %	1·20	1·31[1]	1·66	1·85

[1] One analysis only.

These results demonstrate the increase in phosphorus in the successive extractions. They show the highest phosphorus percentage of our protagons (Posner and Gies obtained also products with 1·73 per cent. phosphorus). The products obtained by the last two series of extractions were now subjected to recrystallisation from boiling acetone and gave the following results.

Phosphorus percentage in fractional products obtained by recrystallisations from acetone.

Original protagon	1·66	1·85
Recrystallised protagon	0·97	1·06[1]
Insoluble protagon	2·01	2·18[1]

[1] One analysis only.

The figures obtained on analysis justify our former remarks.

Finally we subjected some protagon prepared in the same way to recrystallisation from the above mentioned mixture of chloroform and absolute alcohol (or methyl-alcohol). The original protagon contained 1·39 % phosphorus, while in both cases the recrystallised substance showed only 0·40 per cent. phosphorus.

Optical Activity of Protagon.

The optical activity of protagon has not, so far as we are aware, been examined. We found it to be dextro-rotatory. The estimations were made in a 2 dm. tube in a Schmidt and Haensch half-shadow apparatus for sodium light. The strength of the solutions was approximately 5 per cent. and a mixture of three volumes chloroform with one volume

methyl-alcohol was used as a solvent. The readings had to be taken at 28°—30° on account of the insolubility of some of the substances at lower temperatures. We examined several of the protagon preparations described above and the results are given in the following table.

Specific rotatory power of protagon.

	P%	$[\alpha]_D$
Couerbe's cérébrote (thrice recrystallised)	0·65	+6·8°
Cramer's crude protagon	1·02	+5·6°
,, ,, ,, thrice recrystallised	0·79	+7·1°
Fraction of above insoluble in acetone	0·96	+6·3°
Gamgee and Blankenhorn's protagon, twice recrystallised, insoluble in acetone	1·05	+6·1°
Protagon prepared by acetone extraction (see Table IX.).		
2nd fraction at 0°	0·82	+2·7°
3rd fraction at 30°	1·54	+7·5°
4th fraction at 0°...	1·06	+5·1°

As will be seen from these figures, the specific rotatory power of different preparations varies largely, the lowest being +2·7° and the highest +7·5°[1]. These results again demonstrate the composite nature of protagon.

We have also made experiments on the amount of galactose formed by acid hydrolysis of protagon. The amount of galactose was estimated polarimetrically and gravimetrically (by the Allihn-Kjeldahl method). These experiments, which will be fully communicated in another paper, show that the amount of galactose split off from protagon of different preparations varies from 8 to 16 per cent.

In the course of these hydrolytic experiments it was also found that the insoluble products of decomposition included the hydrochlorate of a base which in its properties closely resembles the base sphingosine, isolated by Thudichum from phrenosin. Liebreich (*loc. cit.*) obtained choline (originally called by him neurine) from protagon by hydrolysing it with baryta water, and Cramer (*loc. cit.*) subsequently concluded that "by the decomposition of protagon with baryta water choline is the only base formed." On repeating Liebreich's and Cramer's experiments and decomposing protagon with baryta water, we were also able to isolate sphingosine, which is present in considerable quantity amongst the insoluble hydrolytic products. Of these the bases have been neglected

[1] It is interesting to note that Kitagawa and Thierfelder found under similar conditions the specific rotatory power of cerebron (phrenosin), the main phosphorus-free constituent of protagon, to be +7·6°.

by Liebreich and Cramer, who only examined the watery solution, from which choline may be easily isolated. Choline is probably derived entirely from the phosphorised constituents of protagon (such as Thudichum's sphingomyelin), and part at least of the sphingosine owes its origin to the phrenosin moiety. The isolation from pure protagon of sphingosine, which has been completely overlooked by previous workers, affords evidence for the complexity of the constituents of the mixture called "Protagon."

Conclusions.

1. Liebreich's, Gamgee and Blankenhorn's and Cramer's protagons represent practically the same substance as cérébrote prepared by Couerbe in 1834.

2. A similar substance is obtained by the acetone method described above.

3. All these protagons may be split into substances of widely varying phosphorus and nitrogen percentage by simple fractional crystallisation at different temperatures or with different solvents, showing great differences in their optical activity and in the amount of galactose split off by acid hydrolysis.

4. The base sphingosine, as well as choline, is found amongst the products of protagon-hydrolysis.

5. Protagon (with about 1 % phosphorus) is not a definite chemical compound, but consists of a mixture of substances, some phosphorus-free (like phrenosin), others rich in phosphorus (like sphingomyelin).

6. The use of the term "Protagon" has only a historical justification.

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Note at correction of proof. Since this paper was sent to press, a short communication on "Protagon" by A. C. Lockhead and W. Cramer has appeared in the *Biochemical Journal* (II. p. 350. 1907). These authors come to the conclusion that "the close agreement between the phosphorus percentage of various samples of protagon prepared by the most diverse methods is strong evidence in favour of the view that protagon is an individual substance of a well-defined chemical composition," and that

"the view that protagon is a mixture of substances...cannot be accepted until the substances constituting the mixture have been isolated." Their first conclusion we consider to be completely disproved by our work. With regard to their second statement we may add that since our paper was finished, we have been able to separate protagon into its main constituents, and we are at present engaged in examining and analysing them. The method employed is the one already indicated of systematic fractionation by means of alcohol-chloroform-acetone. The largest fraction (approximately 60—70 per cent. of the original protagon) comprises at least two crystalline substances which are nearly phosphorus-free (P = 0·09 %). The quantity of phosphorus in the remaining substances (of which one so far has been obtained in crystalline form) amounts to about 3 %. It is evident that a mechanical mixture of these substances in the above proportions would enable us to artificially reconstitute "a pure protagon" with a phosphorus percentage varying from 0·9 to 1·2. Full details will be published later.